Rejection under 35 USC 112, first paragraph:

Claims 11-14, 24, 26, and 29-31 are rejected under 35 USC 112, first paragraph on the basis that they are unsupported by the specification. More particularly, it is objected that there is no support in the specification for the concept of altering a naturally-occurring protein's amino acid sequence by replacing amino acids with cysteine residues or by inserting cysteine residues into the amino acid sequence so that a derivative of the naturally-occurring protein can be synthesized by the disclosed method. More generally, it is alleged by the Examiner that there is no support in the specification for derivatives of naturally isolatable proteins containing one or more variant residues that are not found in the naturally isolatable protein.

Applicant traverses this basis for rejection. Scheme 9 on page 39 (now Figure 9) illustrates the synthesis of a mutant form of HIV-1 protease ("Mutant HIV-1 'K41' Protease"), wherein the naturally occurring lysine at position 41 is replaced by a cysteine. More particularly, Scheme 9 illustrates the insertion of cysteine at position 41 within the peptide segment 41-99 and the subsequent ligation of the 41-99 peptide segment to the 1-40 peptide segment by means of the claimed ligation process so as to form the "Mutant HIV-1 'K41' Protease" of length 1-99, wherein cysteine substitutes for lysine (K) at position 41.

Withdrawal of this ba7sis for rejection is respectfully requested.

Rejection under 35 USC 112, second paragraph:

Claims 24, 26, and 29-31 are rejected under Rejection under 35 USC 112, second paragraph as vague. More particularly, it is alleged that the term "an intermediate conformation" is indefinite. Applicant traverses this basis of rejection. However, Applicant has amended 24 so as to introduce structural limitations that clarify the meaning of the term "intermediate." Applicant's amendments obviate this basis of rejection. Withdrawal of this basis of rejection in view of Applicant's amendments is respectfully requested.

Nonstatutory Double Patenting:

Claims 8 and 10-14 have been rejection on the basis of Nonstatutory Double Patenting. A terminal disclaimer has been submitted herewith. Withdrawal of this basis of rejection is respectfully requested.

Rejection under 35 USC 102(b):

Claims 24, 26, and 29-31 have been rejected as anticipated by Yamagishi et al (U.S. Patent No. 4,990,455). Yamagishi discloses a mutant protein wherein a naturally occurring amino acid having a neighboring non-β-branceh amino acid residue has been replaced by cycteine. Applicant's amendment to claim 24 redirects the claim to a key intermediate of the ligation process. Yamagishi does not disclose this key intermediate. Withdrawal of this basis of rejection is respectfully requested.

Summary:

Claims 8, 10-14, 24, 26 and 29-32 are pending. Claims 24, 26 and 29-31 have been amended. Claim 32 has been newly added. Claims 8, 10-14, 24, 26 and 29-32 are clear, fully supported by the specification, and unanticipated by the cited prior art. Allowance of Claims 8, 10-14, 24, 26 and 29-32 is respectfully requested.

Respectfully submitted,

10/17/03
Date

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1. (cancelled) A method for ligating a first oligopeptide with a second oligopeptide end to end for producing an oligopeptide product, the method comprising the following steps:

Step A: admixing the first and second oligopeptides in a reaction solution including a catalytic thiol, the first oligopeptide including a C-terminal thioester, the second oligopeptide including an N-terminal cysteine having an unoxidized sulfhydryl side chain; then

Step B: condensing the unoxidized sulfhydryl side chain of the N-terminal cysteine with the C-terminal thioester for producing an intermediate oligopeptide linking the first and second oligopeptides with a β-aminothioester bond; and then

Step C: rearranging the β-aminothioester bond of the intermediate oligopeptide of said Step B for producing the oligopeptide product linking the first and second oligopeptides with an amide bond.

2. (cancelled) A method as described in Claim 1 wherein, in said step A, the catalytic thiol is selected from the group consisting of unconjugated mercaptans and conjugated thiols.

3. (cancelled) A method as described in Claim 2 wherein, in said step A, the catalytic thiol is benzyl mercaptan.

4. (cancelled) A method as described in Claim 2 wherein, in said step A, the catalytic thiol is a conjugated thiol selected from the group consisting of thiophenol, 1-thio-2-nitrophenol, 2-thio-benzoic acid, 2-thio-pyridine, 4-thio-2-pyridinecarboxylic acid, and 4-thio-2-nitro-pyridine.

5. (cancelled) A method as described in Claim 4 wherein, in said step A, the conjugated

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thiol is thiophenol.

6. (cancelled) An oligopeptide intermediate comprising:

a first oligopeptide segment having a C-terminal thioester,

a second oligopeptide segment having a N-terminal cysteine, and

a β-aminothioester linkage unit linking the C-terminal thioester and the N-terminal cysteine, said β-aminothioester linkage unit spontaneously rearranging intramolecularly to form an amide bond linking said first and second oligopeptides segments end to end.

7. (cancelled) A method for producing an oligopeptide having a C-terminal thioester, the method comprising the following steps:

Step A: providing a resin having a linker with an unoxidized thiol;

Step B: providing a Boc-amino acid succinimide ester; then

Step C:: admixing the resin of said Step A and the Boc-amino acid succinimide ester of said Step B under reaction conditions for producing a Boc-amino thioester-resin; then

Step D: assembling an oligopeptide onto the Boc-amino thioester-resin by stepwise solid phase peptide synthesis; then

Step E: cleaving the Boc-amino thioester-resin of said Step D with HS for producing an oligopeptide having a C-terminal thiol; and then

Step F: converting the oligopeptide having a C-terminal thiol of said Step E to the oligopeptide having a C-terminal thioester.

8. (previously amended) A method for producing a desired protein or domain thereof, which comprises admixing:

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(I) a first oligopeptide, said first oligopeptide comprising a fragment of said desired protein or domain thereof, and having a C-terminal thioester; and

(II) a second oligopeptide, said second oligopeptide comprising a fragment of said desired protein or domain thereof, and having an N-terminal cysteine amino acid residue having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with said C-terminal thioester that rearranges to form an amide bond therein between;

wherein said admixing is conducted under conditions sufficient to permit the formation of an amide bond between the C-terminus of said first oligopeptide and the N-terminus of said second oligopeptide.

9. (cancelled) The method of claim 8, wherein said N-terminal amino acid residue is a cysteine residue.

10. (previously added) The method of claim 8, wherein said desired protein is a naturally isolatable protein.

11. (previously amended) The method of claim 8, wherein said desired protein is a derivative of a naturally isolatable protein that contains one or more variant residues that are not found in said naturally isolatable protein.

12. (previously added) The method of any of claims 10 or 11, wherein said protein is a mammalian protein.

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13. (previously added) The method of claim 12, wherein said mammalian protein is a human protein.

14. (previously added) The method of claim 13, wherein said human protein is a cytokine.

15. (cancelled) The method of claim 8, wherein in said method said second oligopeptide has a C-terminal thioacid, and wherein said method additionally comprises the steps of:

(A) converting said thioacid to a thioester; and

(B) admixing said converted thioester with a third oligopeptide, said third oligopeptide comprising a fragment of said desired protein or domain thereof, and having an N-terminal amino acid residue having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with said C-terminal thioester that rearranges to form an amide bond therein between, wherein said admixing is conducted under conditions sufficient to permit the formation of an amide bond between the C-terminus of said second oligopeptide fragment and the N-terminus of said third oligopeptide fragment.

16. (cancelled) The method of claim 15, wherein said N-terminal amino acid residue is a cysteine residue.

17. (cancelled) A synthetically produced protein of greater than about 35 amino acid residues, wherein all of the residues of said protein are linked to adjacent residues via an amide bond.

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18. (cancelled) The synthetically produced protein of claim 17, wherein said protein has greater than about 70 amino acid residues.

19. (cancelled) The synthetically produced protein of claim 17, wherein said desired protein is a naturally isolatable protein.

20. (cancelled) The synthetically produced protein of claim 17, wherein said desired protein is a derivative of a naturally isolatable protein that contains one or more cysteine residues that are not found in said naturally isolatable protein.

21. (cancelled) The synthetically produced protein of any of claims 19 or 20, wherein said naturally isolatable protein is a mammalian protein.

22. (cancelled) The method of claim 21, wherein said mammalian protein is a human protein.

23. (cancelled) The method of claim 22, wherein said human protein is a cytokine.

24. (presently amended) A synthetically produced protein intermediate having ~~of~~ greater than about 35 amino acid residues, ~~said protein having an intermediate conformation, wherein all of the residues of said protein are linked to adjacent residues via an amide bond,~~ said protein being produced by ~~the~~ a process of ligating together a first oligopeptide fragment and a second oligopeptide fragment, ~~at least two oligopeptide fragments wherein:~~

~~(1)~~ said first oligopeptide fragment having a length of 30 or more amino acid residues ~~with a C-terminal non-β-branched amino acid residue modified~~

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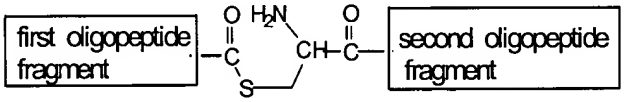
~~as a C-terminal thioester; and~~

~~(2) said second oligopeptide fragment has an *N*-terminal cysteine having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with said C-terminal thioester that rearranges to form an amide bond therein between; wherein said ligation results in the formation of an amide bond linking said first and second fragments, wherein said synthetically produced protein being a derivative of a naturally isolatable protein or fragment thereof, said *N*-terminal cysteine not being found in the naturally isolatable protein;~~

~~the intermediate conformation of said protein resulting directly from said ligation process.~~

said synthetically produced protein intermediate being represented by the following structure:



25. (cancelled) The synthetically produced protein of claim 24, wherein said N-terminal amino acid residue is a cysteine residue.

26. (presently amended) The synthetically produced protein intermediate of claim 24, wherein said protein intermediate has greater than about 70 amino acid residues.

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27. (cancelled) The synthetically produced protein of claim 24, wherein said desired protein is a naturally isolatable protein.

28. (cancelled) The synthetically produced protein of claim 24, wherein said desired protein is a derivative of a naturally isolatable protein that contains one or more cysteine residues that are not found in said naturally isolatable protein.

29. (presently amended) The synthetically produced protein intermediate of Claim 26, wherein said synthetically produced protein intermediate being intermediate to ~~naturally isolatable protein is~~ a mammalian protein.

30. (presently amended) The synthetically produced protein intermediate of claim 29, wherein said mammalian protein is a human protein.

31. (presently amended) The synthetically produced protein intermediate of claim 30, wherein said human protein is a cytokine.

32 (new) A method for producing a desired protein or domain thereof, which comprises admixing:

(I) a first oligopeptide, said first oligopeptide comprising a fragment of said desired protein or domain thereof, and having a C-terminal thioester; and

(II) a second oligopeptide, said second oligopeptide comprising a fragment of said desired protein or domain thereof, and having an N-terminal cysteine amino acid residue having an unoxidized sulfhydryl side chain and a free amino group that is capable of forming a β-aminothioester linkage with

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said C-terminal thioester that rearranges to form an amide bond therein between;

wherein said admixing is conducted under conditions sufficient to permit the formation of an amide bond between the C-terminus of said first oligopeptide and the N-terminus of said second oligopeptide;

wherein said desired protein is a derivative of a naturally isolatable protein, said desired protein containing one or more cysteine residues that are not found in said naturally isolatable protein.